International Journal of Applied and Pure Science and Agriculture
ISSN Online - 2394-5532 Impact Factor : 4.446 by SJIF ISSN Print - 2394-823X

Phytochemical analysis and antifungal activity of Cauliflower stem (*Brassica oleraceae var botrytis* L.)

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Abstract
Nature has provided a complete store house of remedies to cure all ailment of mankind. The present study was carried out to investigate the phytochemicals profile of stems of Brassica oleraceae. The stem powder was successively extracted with petroleum ether, chloroform and ethanol. Phytochemicals analysis showed the presence of alkaloids, flavonoids, steroids, terpenoids, anthraquinone, protein phenols, Quinone and carbohydrate. The result of the study could be useful for human therapy, veterinary, agriculture and scientific research of the plant. Antifungal activity of Brassica oleracea stem against Aspergillus niger was carried out and it was found that the extracts have maximum percentage of growth inhibition against Aspergillus niger.
Keywords: Phytochemicals, Aspergillus niger, Steroids, Brassica oleracea.

I. INTRODUCTION

Phytochemicals are bioactive chemicals, synthesized naturally in all parts of the plant body [12]. The quantity and quality of phytochemicals present in plant parts may differ from one part to another. Secondary metabolites are known to show curative activity against several elements in man and other animals and the use of traditional medicinal plants for treatment of several diseases.

The phytochemicals for their physiological role in plants, such as in stress response and resistance to diseases and their ability to increase agronomic performances. Among vegetables, cauliflower (*Brassica oleraceae* L.) is particularly rich in phytochemicals, such as glucosinolate, vitamin C, polyphenols and glucosinolates are formed by a glycone are hydrolyzed to glucose and isothiocyanates by myrosinase [6].

The development of microbial resistance towards antibiotics has heightened the importance of the search for new potential effective plants and plant constituents against pathogenic microorganisms [1]. Antimicrobial screening of plant extracts and the phytochemical represent a starting point for antimicrobial drug discovery [5]. The present study reports on the phytochemical analysis and antimicrobial activities of various extracts from *Brassica oleracea* stem.

II. MATERIAL AND METHODS

2.1. Collection and identification of plant material

B. oleracea were collected in and around markets of Coimbatore, Tamil Nadu, India. The authenticity of the plant was confirmed in Botanical Survey of India, Tamil Nadu Agricultural University, Coimbatore. The *B. oleracea* stem were washed with water to remove the dirt and shade dried. The shade dried samples were powdered separately using an electrical grinder. The powder was stored in screw cap bottles until further analysis.

2.2. Extraction proceed

The 10g of *B. oleracea* stem powder was weighed using an electrical balance (Denver 210) and made into 8 packets using zerohaze filter paper (10 A grade SD'S). These powders were subjected to extraction with 500 ml of the solvents for 8 h using a Soxhlet apparatus [7, 13]. Petroleum ether (60-80^{0}C) extraction was followed by chloroform extraction and ethanol extraction so that the powders were subjected to extraction with solvents of increasing polarity.

The stem extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath at 40^{0}C. The residue thus obtained was stored in tightly closed glass vials in the refrigerator for further use.

Figure 1 Cauliflower Stem

2.3. Phytochemical screening

The phytochemicals screening of *B. oleracea* stem extracts were carried out to determine the presence of the following compounds alkaloids, phenols, flavonoids, terpenoids, steroids, anthraquinones, proteins, quinines and carbohydrate using the colour test adapting standard methods [10].

2.4. Test microorganism and Antifungal assay

The fungal strain used was *A. niger* and the activities of the plant extracts on fungal strain was assayed by agar well diffusion method.

2.5. Agar well diffusion method

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters. The commercially available (Hi media) Rose Bengal Chloramphenicol agar medium (32.15 g) was suspended in 1000 ml distilled water. The medium was dissolved completely by boiling and was then autoclaved at 15 lbs pressure (121^{0}C) for 15 minutes.

2.6. Agar Medium Preparation

Rose Bengal Chloramphenicol agar medium was prepared and poured on to the petriplates. A fungal plug was placed in the centre of the plate. Wells 6 mm diameter was cut into the agar medium. Petroleum ether, chloroform and ethanol extract of the plants was poured onto the wells in the plates. Nystatin was used as antifungal control and DMSO as negative control. The antifungal effect was seen as crescent shaped zones of inhibition [11].

2.7. Statistical analysis

The antimicrobial data was assayed by standard deviation by mean of three replicates.

III. RESULT AND DISCUSSION

In the present investigation, the phytochemical screening of extract of *B. oleracea* stem showed presence of phytochemicals constituents. The results confirmed the presence of constituents which are

known as physiological activities. Phytochemical constituents such as tannins, flavonoids, alkaloids, phenol, terpenoids, coumarins and several other aromatic compounds are regarded as secondary metabolites of plants [3]. The present study was carried out on the various plant extract to reveal the presence of medicinally active constituents such as alkaloids, flavonoids, steroids, terpenoids, anthraquinone, , protein, phenols, quinone and carbohydrate in the selected plant which could be responsible for the observed antimicrobial property.

The result obtained in the phytochemical analysis of petroleum ether extracts showed the presence of alkaloids, flavonoids, anthraquinone and carbohydrate. In chloroform extract alkaloids, flavonoids, steroids, anthraquinone, protein, phenols and carbohydrates were present. Presence of alkaloids, flavonoids, anthraquinone, protein, phenols, Quinone, carbohydrate and quinone were in the ethanol extract of *B. oleracea* stem (Table-1).

Table 1. Phytochemical constituents of cauliflower stem

S.No.	Test		Pet. Ether	Chloroform	Ethanol
1	Alkaloids	Mayers	-	-	+
		Wagners	+	+	-
		Hagers	+	+	+
2	Flavonoids	i) Sod.Hydroxide test	-	-	+
		ii) Sulphuric acid test	+	+	-
3	Steroids	Libermann-Burchard	-	+	-
4	Terpenoids	Libermann-Burchard	-	-	-
5	Anthraquinone	Borntragers	+	+	+
6	Protein	i) Ninhydrin (Aq)	-	-	+
		ii) Ninhydrin (Acetone)	-	-	+
		iii) Biuret	-	+	-
7	Phenols	i) Ferric Chloride	-	+	+
		ii) Libermann	-	+	+
8	Quinone	ConcHCl test	-	-	+
9	Carbohydrate	i) Molish	+	+	+
		ii) Fehlings A &B	+	-	+

\+ Detected - Not detected

The phytochemical diversity of antimicrobial compounds include terpenoids, saponins, phenolics and phenyl propanoids, stilbenes, alkaloids, glucosinolates, hydrogen cyanide, indole and also elements sulphur, the sole inorganic compound [4]. The alkaloids contained in plants are used in medicine as anesthetic agents [8]. The presence of saponins in plants have been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs [2].

The potential for developing antimicrobials from plants appear rewarding and the development of a phytomedicine to act against microbes. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials [9].

In the present study, the agar well diffusion method was used to evaluate the antimicrobial activity by measuring the inhibition zone against the test microorganisms. The results obtained by various extracts of *B. oleracea* stem showed that petroleum ether extract exhibited antifungal activity

against *A. niger*. The extract of petroleum ether extract showed that maximum inhibition zones against test organisms (Fig 2&3).

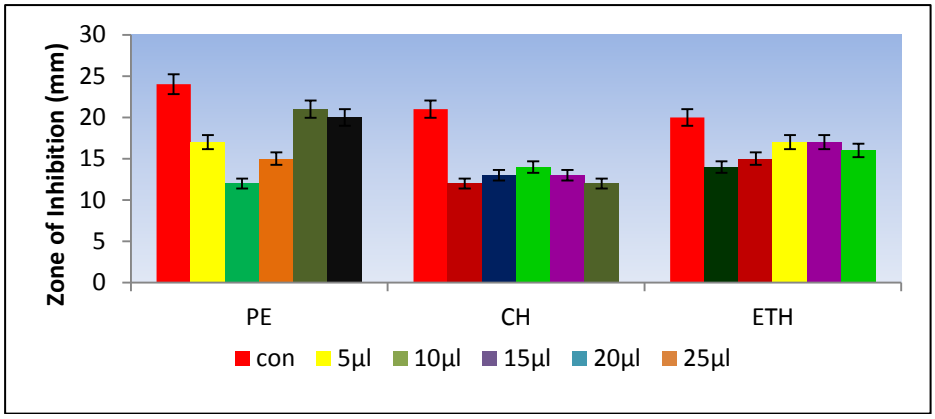

Figure 2 Antifungal activity of stem of Brassica oleracea extract against test organisms

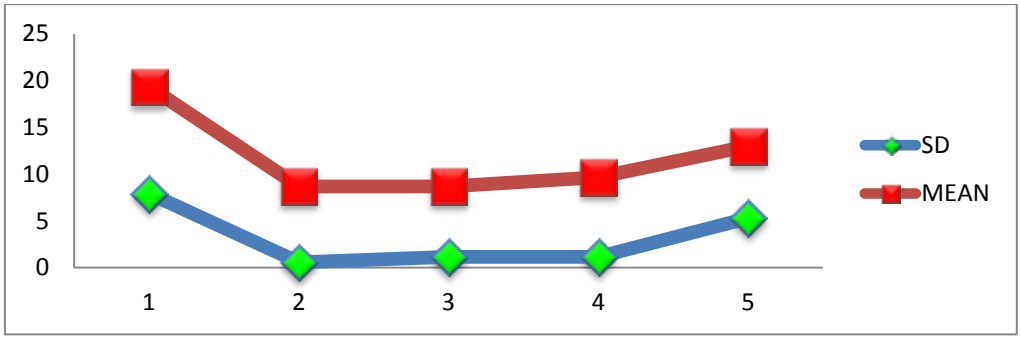

Figure 3 Antifungal activity of Aspergillus niger

V. CONCLUSION

The present study revealed that a number of phytochemicals were found which is beneficial for the human health. The antimicrobial nature of these plants could be used to improve natural antimicrobial drug from cauliflower.

VI. ACKNOWLEDGEMENT

This work was carried out with the support of Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, TamilNadu, India.

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